Original Research Article

Impact of Spent Mushroom Substrate Integration with *Trichoderma harzianum* on Yield and Gummosis Disease Incidence in Bottle gourd (*Lagenaria siceraria*)

ABSTRACT

Bottle gourd is a commonly grown cucurbitaceous vegetable crop in India. Its fruits are traditionally used in the treatment of many diseases. The area and production of bottle gourd in India is 1.93 lakh ha, 3.17 million tonnes, respectively whereas in Haryana it is cultivated on 20850 ha. with production of 3.03 lakh tonnes per year (Anonymous, 2021). It is affected by various pathogenic fungi , bacteria, viruses and nematodes but gummosis, a soil borne fungal disease is a serious threat in bottle gourd production in Haryana. The initial symptoms are change in color from normal to reddish brown at the collar region as well as change in plant posture from erect to trailing one. The longitudinal splitting and oozing of brownish gum on the discolored stem is the first sign of the disease. The infection spread upwards and subsequently plant wilts. This disease causes a considerable loss in bottle gourd production and productivity. The demand for organic residues and compost has increased several times in recent years because of promotion of organic farming and mitigation of inorganic fertilizers, pesticides etc. The research work carried out around the world has proved that spent mushroom substrate (SMS) possesses the quality of good organic manure for raising healthy crops. Although farm yard manure (FYM) is mostly being used for production of organic foods previously but due to its poor availability, an alternate organic nutrient source is the application of SMS in agricultural crops. Its application in soil after proper removing of soluble salts and weathering for at least one year has been found to increase the yield and quality of different vegetable crops. Its application in soil has also been found to decrease in disease incidence in different vegetable crops. Keeping in view of its nutritional importance, lack of FYM availability and increase of SMS production in future, it was felt to study the impact of soil application of SMS and FYM alone as well as its integration with *T. harzianum* on bottle gourd seed germination, yield and gummosis disease incidence. The twelve months old SMS and well rotten FYM were applied in soil alone as well as in integration with *T. harzianum* in the experiments conducted for three years during 2019 to 2021. Among the various treatments, the application of SMS and FYM applied alone or enriched with *T. harzianum* didn't have any adverse effect on bottle gourd germination. However, the maximum average of bottle gourd yield obtained was 5752 kg/ha in SMS enriched with *T. harzianum* followed by 5630 kg/ha in soil application of SMS alone and a minimum average yield of 4806 kg/ha was obtained in control. It was also observed a minimum average of gummosis disease incidence of 4.8 per cent when SMS enriched with *T. harzianum* was applied in soil before sowing and it was followed by 6.5 per cent when SMS was applied alone. The maximum average of gummosis disease incidence (11.7 per cent) was observed in control.

Keywords: Bottle gourd, disease incidence, farm yard manure, spent mushroom substrate, yield.

1. INTRODUCTION

Bottle gourd is one of the most commonly grown cucurbitaceous vegetable crops in India. It is grown in warmer regions of the world. It is now popular for its several health benefits and easy digestion. It has high yield potential and steady market price. Its fruits are being used in the treatment of human diseases like jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure, skin diseases, heart diseases etc. It is rich in iron, vitamins, potassium and contains various flavonoids, saponins, triterpenes, and volatile principles. It is an important gift from Ayurveda to mankind (Prajapati *et al*. 2010) and widely cultivated in India, Sri Lanka, China, and many parts of the world. In India, it was cultivated on an area of 1.93 lakhs ha with 3.17 million tonnes production during 2020-21 (Anonymous, 2021). It is being widely cultivated in Bihar, U.P., M.P., Haryana, Chhatisgarh etc., however, it was cultivated on an area of 20850 ha. with production of 3.03 lakh tonnes during 2020-21 in Haryana.

It is affected by various diseases like gummosis, Alternaria leaf blight, anthracnose, Cercospora leaf spot, downy mildew, scab, powdery mildew, Septoria leaf spot, Verticillium wilt, angular leaf spot, aster yellows, cucumber mosaic virus etc. The gummosis is a threatening disease of bottle gourd in Haryana caused by a soil borne fungus *Cladosporium cucumerinum*, which overwinters in debris. The initial symptoms of the disease are change in normal to reddish brown colour at the collar region and change in plant posture from erect to trailing one. The oozing of brownish gum on the discolored stem is the first sign of disease. The infection spreads upward and plant wilts which cause a considerable loss in its production and productivity.

There is a considerable increase in demand of FYM to reduce the use of inorganic fertilizers, pesticides etc. and previously it is being mostly used for production of organic foods but due to its poor availability, SMS is a substitute in raising organic crop produce. It is considered as a waste after mushroom harvest and its piling up causes various environmental problems, like ground water contamination, nuisance etc. (Beyer, 1996). The mushroom production is being increased every year and presently being left with an approx. of 6 lakh tons SMS annually. Zang *et al*., 1995 reported that SMS has crude protein, fibre and ash contents. In addition, it has high cation exchange capacity, slow mineralization rate, 45% water and is light in weight (Dann, 1996). It contains N (1.9%), P (0.6%) and K (1.0%) after 8-16 months weathering (Gupta *et al*., 2004) and an EC (1.9 to 8.3 memos/cm), pH of 7.28 (Wuest and Fahy, 1991). Keeping in view of its physical, chemical and biological properties, research work is being conducted in many countries on its uses in vegetables, horticultural and field crops production. The removal of soluble salts and weathering before application is an important task before its soil application. The soil application of SMS has been found to increase yield, improved product quality and decreased soil borne diseases in crops. Ahlawat and Sagar (2007) revealed that the soil application of SMS when used at 18.5 tonnes/ha in tomato influenced superior fruit weight, high ascorbic acid content, high dry matter, more total soluble solids (TSS) etc. and decreased incidence of soil borne diseases. The results have also been found to be encouraging in chilli (*Capsicum annuum*), pea (*Pisum sativum*), cauliflower (*Brassica oleracea* var *botrytis*), ginger (*Zingiber officinale*), onion (*Allium cepa* L.), brinjal (*Solanum melongena*) etc. Keeping in view of its importance, shortage in FYM availability, environmental issues in future, it was felt to study the impact of soil application of SMS as well as FYM alone as well as their integration with *T. harzianum* on bottle gourd yield and soil borne disease incidence of gummosis in the field experiments conducted consecutively for three years from 2019 to 2021.

2. MATERIALS AND METHODS

The experiment to study the impact of soil application of SMS and FYM alone and their integration with *T. harzianum* on yield and gummosis disease incidence of bottle gourd cv. Pusa Summer Prolific Long (PSPL) was carried out in soil naturally infested with gummosis pathogen in the experimental field area of Department of Plant Pathology, CCSHAU Hisar from 2019 to 2021. The one year naturally weathered button mushroom SMS and two years old well rotten FYM was used in the experiment. The pure culture of *T. harzianum* was collected from Biological Control Lab., Department of Plant Pathology, Chaudhary Charan Singh Haryana Agricultural University, Hisar and multiplied on oatmeal medium under *in vitro* conditions as per standard procedure. The oatmeal medium containing *T. harzianum* (1 x 10^9 cfu/g) was mixed at the rate of 0.001 per cent (w/w basis) in thoroughly wet SMS and FYM in shade and covered with gunny bags for 15 days. The water was sprinkled for 15 days on daily basis upon gunny bags to favor the multiplication of *T. harzianum* in both substrates. After 15 days each substrate was made uniform by mixing with hands. The SMS and FYM without *T. harzianum* integration were also maintained similarly. The soil application of substrates was carried out as per treatments given below and sowing of bottle gourd cv. PSPL was done on sides of raised beds adjacent to irrigated channel at a spacing of 60 cm from plant to plant in mid February during all the three years. The plot size of treatment was kept as 5 m × 3.3 m. Each treatment was replicated thrice and randomized completely. The agronomic operations were carried out as per recommended package of practices. The plots without soil amendments were also maintained as per recommended package of practices and served as control. The observations were taken on seed germination (%), yield (kg/ha) and gummosis disease incidence (%) till the end of crop season. The yield data was compiled replication wise at the end of the season. The gummosis incidence was also recorded replication wise at a weekly interval after germination of seedlings. The experiment was repeated for three consecutive years from 2019 to 2021. The sowing of the bottle gourd was done in the mid February of every year. The data were analyzed statistically by randomized block design through OPSTAT software.

Treatments:

T_1= 25 tonnes SMS amended with *T. harzianum* /ha
T_2= 25 tonnes SMS alone/ha
T_3 =25 tonnes FYM amended with *T. harzianum* /ha
T_4= 25 tonnes FYM alone/ha
T_5= Control (No soil amendment with SMS or FYM)

3. RESULTS AND DISCUSSION

None of the treatment, affected seed germination. However, soil application of SMS enriched with *T. harzianum* resulted in a significant higher yield of 6125 kg/ha as compared to 5000kg/ha in control during 2019. The maximum average yield was 5752 kg/ha in SMS enriched with *T. harzianum* followed by 5630 kg/ha in soil application of SMS alone and a minimum average yield of 4806 kg/ha in control (Table 1). In other words, there was an increase in yield of 19.5 per cent in SMS enriched with *T. harzianum* followed by 17.0 per cent when SMS was applied alone. The increase in yield was 10.1 per cent when FYM enriched with *T. harzianum* was applied in soil followed by 7.2 per cent when FYM was applied alone as compared to control (Fig. 1). Daniel *et al* (2021) based on his findings revealed that after proper composting of SMS it should be reintroduced into a production system as a promising organic growing media that produced higher red baby leaf lettuce yields. This study corroborate with our finding that application of SMS in soil gave higher yield. Prasad *et al*. (2021) investigated that fresh SMS influenced yield, leaf area, fresh-dry plant weights and found it as an effective and inexpensive peat substitute in 15 and 25% (v/v) in straw berry cultivation. biomass production (fresh mass, dry mass, root length, fresh root mass, and dry root mass) and sensory evaluation were determined 35 days after planting. Muchena *et al*. (2021) showed that increasing rates of SMS significantly increased the fresh yield and quality of baby spinach but its application beyond 30 tonnes/ha increased the bitterness of baby spinach. The results of Marica *et al.* (2015) also corroborate our findings that SMS is a good substrate for cultivation of *Trichoderma* and in addition they also gave information that *Trichoderma* produces excess of enzymes like cellulase, xylanase, amylase and β-glucosidase in SMS without any supplementation. Singh *et al.* (2018) also evaluated the impact of SMS enriched with *T. harzianum* on natural antioxidant and nutritional properties of tomato and revealed that it led to a significant increase in natural antioxidants, minerals, total polyphenol, flavonoid, Fe^{2+}/Fe^{3+} chelating activity, superoxide anion radical scavenging activity in tomato which is highly desirable for human health. Roy *et al.* (2015) found that application of SMS in soil led to the overall increase in growth of *Capsicum annuum* L. and an increase in carotenoid, protein (2.5 times) over control. Sagar *et al*. (2009) reported that soil application of SMS increased yield in Capsicum, tomato, cauliflower, ginger, garlic, wheat, maize, paddy, potato, pea and apple. The amendment of soil with SMS at 18.5 tonnes/ha alone led to a higher yield of 746q/ha in tomato as compared to 456.53q/ ha in soil application of FYM alone (Ahlawat and Sagar, 2007). These studies are in line with our findings that its application produced higher yield in bottle gourd throughout three years of experimentation. Chong *et al*., 1991 and Beyer, 1996 studies revealed that leaching of salts and its weathering for two to three years is essential for growing vegetables and fruits of economic importance. Wisniewska and Penkiewicz (1989) while working on soil application of SMS @ 50 tonnes/ha in onion found that inspite of increase in yield it also improved P, K, Ca and Mg contents in bulbs. In the present study, SMS enriched with *T. harzianum* when applied as soil treatment before sowing of bottle gourd led to a synergistic increase in yield, probably it may be due to its better organic nutritional status, antagonistic effect of SMS microflora, better establishment of *T. harzianum* which might have acted against various soil borne pathogens and hence higher yield. This finding is being supported by many studies as described above with an indication that SMS enrichment with different biocontrol agents and its soil application impact may be tested in other vegetables, horticultural and field crops for yield, nutritional status of produce.

The gummosis incidence was significantly low in the treatment when SMS enriched with *T. harzianum* was applied in soil during all three years of experimentation as compared to control (Table 1). An average of gummosis disease incidence of 4.8 per cent was lowest when SMS enriched with *T. harzianum* was applied in soil before sowing followed by 6.5 per cent when SMS was applied alone in soil. The maximum average of gummosis disease incidence of 11.7 per cent was observed in control. In other words, a maximum of gummosis disease control was 45.9 per cent when SMS enriched with *T. harzianum* was applied in soil followed by 36.0 per cent when SMS was applied alone. The FYM enriched with *T. harzianum* gave an average of 29.4 per cent gummosis disease control and a minimum of gummosis disease control of 11.7 per cent was observed in FYM enriched with *T. harzianum* and applied in soil before sowing. The soil application of SMS enriched with *T. harzianum* gave consistently good and uniform results throughout three years of experimentation providing maximum yield and gummosis disease control in bottle gourd. The SMS nutritional status, microflora and further its enrichment as well as establishment of *T. harzianum* might have produced an additional antagonism against gummosis pathogen and hence, it may be the reason of higher gummosis disease control and consequently higher yield of bottle gourd. Daniel *et al* (2021) revealed that SMS application in a production system gave suppressive effect against *Pythium irregulare* but incorporation of *T. harzianum* did not affect against *P. irregulare*. This study corroborate with our finding that application of SMS alone in soil acted against the gummosis pathogen and hence low disease incidence as compared to control. But in contrast they reported that enrichment of *T. harzianum* did not have additional effect against pathogen, it may be due to variations in *T. harzianum* isolates as well as differences in their SMS microbial community which might have affected the establishment of *T. harzianum*. In a similar study, the wet root rot of chickpea caused by *Rhizoctonia solani* was reduced effectively by soil application of FYM enriched with *Trichoderma* (Jambhulkar,

2015) and this study corroborates with our findings. Gupta et al. (2006) and Ahlawat and Sagar, (2007) have also shown that SMS harbor different microflora like *Pseudomonas, Bacillus, Trichoderma* sp., *Aspergillus* sp., *Mucor* sp., actinomycetes. The presence of microflora on SMS might have resulted in low gummosis incidence in bottle gourd and consequently higher yields. Similarly, the findings of Ahlawat and Sagar, 2007 also support our study that soil application of SMS minimized yield losses due to soil borne pathogens in vegetable crops like tomato, chilli, peas, cauliflower and zinger. Mostafa *et al*. (2019) found that SMS has a nematicidal potential against *M. incognita*, also improved nutritional status and increased tomato yield. Verma, 1986 also noticed the restricted the root knot nematode infestation in tomato by its soil application. Since SMS is a cheapest, abundantly available and environment friendly waste, therefore, trials need to be conducted on its enrichment or establishment with different biocontrol agents and their soil application impact on soil borne pathogens of other vegetables, horticultural and field crops for sustainable crop production.

The substrate analysis was conducted at Department of Soil Science, College of Agriculture, CCS Haryana Agricultural University, Hisar. The data presented in Table 2 revealed that SMS and FYM are approx. at par in P and K concentrations; however, N content is more in FYM (2.5%) as compared to SMS (1.28%). Velusami *et al*. (2021) also analysed covered and uncovered SMS for physical and chemical characteristics after storage for 12 months and found that there were no consistent differences under uncovered or covered storage conditions. The content of nitrogen (N) and manganese (Mn) was significantly lower in uncovered SMS, while the content of iron (Fe) and copper (Cu) was significantly higher. Nitrogen-phosphorus-potassium concentrations per kg wet weight were all higher in SMS that was stored under cover. The high pH of stored SMS ranged between 7.8–8.1 and hence could be used in acid soils and due to its low bulk density (0.545–0.593 g/cm^3) it is an ideal amendment in soils to improve soil structure and quality. Mortada *et al*. (2020) found higher moisture, ash content, pH, and C:N ratio in SMS and the values were 63.00%, 6.58%, 5.92, and 116.29, respectively. Among the nutrients phosphorus, calcium, magnesium, iron, and copper were 57.14 ppm, 7366.67 ppm, 1230.83 ppm, 85.18 ppm, and 3.75 ppm, respectively, meanwhile, the values of nitrogen, potassium, zinc, and manganese were 0.38%, 706.67 ppm, 16.90 ppm, and 68.65 ppm, respectively. In a compiled report of Ahlawat and Sagar, (2007) many physical and chemical properties of SMS have been revealed. They reported that pH of 6.72 was minimum in 9 months weathered to a 7.50 with maximum in 48 months weathered SMS. The electrical conductivity was highest at 6.22 mS cm-1 in 6 months and lowest at 0.24 mS cm-1 in 48 months weathered SMS. The N, P, C, Ca content decreased by increasing weathering duration. The nitrate content in SMS also decreased from 12.80 to 1.95 ppm when it was weathered for 6 and 48 months, respectively. Many of the physical, chemical and biological properties like pH, electrical conductivity, carbon content, micro nutrients, heavy metals, microflora etc. need to be tested before the conduct of trials on weathered SMS, which may really help in substantiating the findings.

4. CONCLUSION

The soil application of SMS enriched with *T. harzianum* resulted in a higher average yield of three years (5752 kg/ha) as compared to control (4806 kg/ha) in bottle gourd. A maximum of gummosis disease control of 45.9 per cent in bottle gourd was achieved in the treatment when SMS was enriched with *T. harzianum* and applied in soil as compared to control.

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Table 1. Effect of soil application of spent mushroom and farm yard manure enriched with *T. harzianum* on germination, yield and gummosis incidence in bottle gourd cv. PSPL

Treatments	*Germination (%)				*Yield (kg)/ha				*Per cent increase in yield				*Gummosis Disease incidence (%)				*Per cent Disease Control			
	2019	2020	2021	Mean	2019	2020	2021	Mean	2019	2020	2021	Mean	2019	2020	2021	Mean	2019	2020	2021	Mean
SMS + TH	90	90	90	90	6125	5970	5161	5752	22.5	21.5	14.5	19.5	05.8	05.8	2.9	4.8	63.1	61.1	13.7	45.9
SMS alone	90	90	90	90	6000	5837	5054	5630	20.0	18.8	12.2	17.0	08.0	08.6	2.9	6.5	49.0	46.9	12.1	36.0
FYM + TH	90	90	90	90	5525	5447	4925	5299	10.5	10.5	9.3	10.1	09.2	09.6	3.1	7.3	41.4	40.7	6.1	29.4
FYM alone	90	90	90	90	5450	5323	4671	5148	09.0	09.0	3.7	7.2	11.3	12.3	3.2	8.9	28.0	24.1	3.0	18.4
Control	90	90	90	90	5000	4913	4505	4806	-	-	-	-	15.7	16.2	3.3	11.7	-	-	-	-
C.D. at 5%	NS	NS	NS	-	135.8	87.4	67.7	-	-	-	-	-	0.57	0.44	0.5	-	-	-	-	-

*Average of three replications

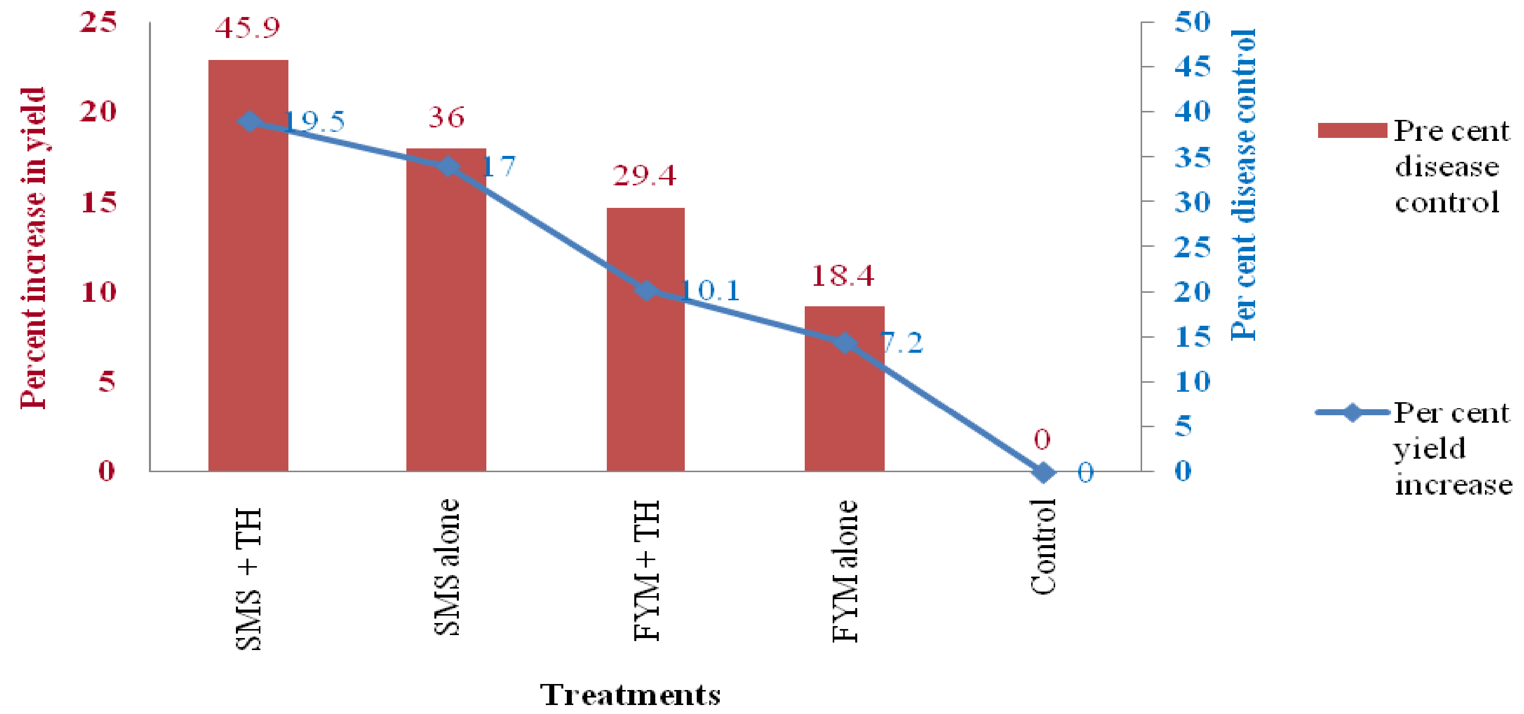

Fig. 1. Impact of soil application of SMS and FYM enriched with *T. harzianum* on yield and disease control in bottle gourd

Table 2. Nutrients status of spent mushroom substrate and farm yard manure

Substrate(s)	**N (%)**	**P (%)**	**K (%)**
Spent Mushroom Substrate	1.28	0.89	1.25
Farm Yard Manure	2.50	0.75	1.56